13

Thyroid Follicles in the Head Kidney of the Goldfish, *Carassius auratus* (Linnaeus)

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(Plate I)

SINCE the positive identification of the thyroid gland in the conger eel, *Conger conger*, by Baber in 1881, this endocrine organ has been identified and described in many species of teleosts. The teleostean gland corresponds closely to that of the Amphibia and amniotes in structure, but is anatomically diffuse and lacks a connective tissue capsule. The individual follicles are more or less scattered in the throat region, most commonly from the first to the fourth aortic arches (Maurer, 1886; Gudernatsch, 1911). In only three groups of teleosts, the swordfish *Xiphias gladius* (Addison & Richter, 1932), the parrotfishes *Pseudoscarus guacamaia, Sparisoma* sp. and *Scarus* sp. (Matthews, 1948), and the mormyrid *Gymnarchus niloticus* (Thomopoulos, 1950), are the follicles grouped into a compact gland with a connective tissue capsule, but even in these species a number of subsidiary follicles may occur outside the main thyroid mass. It is not surprising, therefore, that teleostean tumors of the thyroid usually consist of a tumor body in the throat with invasive growth occurring into the neighboring gill and pericardial regions (Schlumberger & Lucké, 1948; Schlumberger, 1955). Surprisingly, these tumors have only rarely been found to metastasize even when malignant (Nigrelli, 1952; Berg *et al*, 1953; Schlumberger, 1955). Recently, however, Baker *et al* (1955) reported aberrantly-located thyroid follicles and tumors in the platyfish, *Xiphophorus maculatus*. The present study describes the presence of thyroid follicles in the lymphoidal pronephric remnants, the head kidneys, of the goldfish, *Carassius auratus* (Linnaeus), and the responses of these atypically-located follicles to various physiological stimuli.

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MATERIALS AND METHODS

One hundred and fifty-three common, commercially hatched, xanthic goldfish were used in this study, the primary purpose of which concerned melanogenesis (Chavin, 1956). The fish were one to two inches in standard length and less than one year old. They were given dried food (Aronson, 1949) with an occasional supplement of living tubificid worms. During the course of experimental treatment the water temperature ranged from 30° C. in the summer to 22° C. in the winter.

Thirty normal fish were immersed in 0.7% sodium chloride in conditioned aquarium water, and two animals were sacrificed at intervals ranging from one hour to 19 days after initiation of treatment. Thirty control animals were sacrificed at similar intervals. Five hypophysectomized goldfish exposed to 0.7% saline for 14 days and eight hypophysectomized fish maintained under control conditions were also studied. Other experimental groups and controls are indicated in Table 1. The implanted tissues had been freshly removed from large goldfish 8-10″ in standard length. The animals were sacrificed after 10 days of treatment.

The fish were killed and fixed in Bouin's fluid, imbedded in paraffin and sectioned serially at seven microns. The sections were stained with hematoxylin-eosin or Masson's trichrome stain. The epithelial height of the follicles in the head kidney was not measured, because the changes in height were obvious and the follicles were usually too variable in number for such figures to be of statistical significance.

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Table 1. Variation in Thyroid Epithelial Height in Throat and Head Kidney Regions of the Goldfish, *Carassius auratus* L., with Experimental Treatment

Treatment	Number of Animals[1]	Number of Animals with Head Kidney Thyroid	Mean Thyroid (Throat) Epithelial Height[2] *Microns*	Reaction of Thyroid (Head Kidney) Epithelium[3]
Untreated	30N	23	2.29 ± 0.0096	Normal
	8H	7	1.12 ± 0.0124	Atrophy
Immersion in 0.7% Saline	30N	26	1.90 ± 0.0196[4]	Depressed
	5H	4	1.12 ± 0.0145	Atrophy
Pituitary Implant	4N	4	8.33 ± 0.1348	Hypertrophy
	4H	3	8.76 ± 0.1543	Hypertrophy
Optic Lobe Implant	4N	4	1.82 ± 0.0233	Depressed
	4H	2	1.16 ± 0.0207	Atrophy
Head Kidney Implant	4N	4	1.83 ± 0.0215	Depressed
	4H	4	1.15 ± 0.0182	Atrophy
Opisthonephros Implant	4N	4	1.89 ± 0.0240	Depressed
	4H	4	1.15 ± 0.0188	Atrophy
Isotonic Saline Vehicle (0.05 cc. daily)	4N	3	1.90 ± 0.0163	Depressed
	4H	3	1.13 ± 0.0165	Atrophy
ACTH (1 I.U. daily)	4N	4	1.74 ± 0.0192	Depressed
	4H	4	1.12 ± 0.0165	Atrophy
ACTH (1 I.U.) and TSH (0.44 USP unit daily)	4N	3	6.03 ± 0.1045	Hypertrophy
	4H	4	5.90 ± 0.0872	Hypertrophy
ACTH (1 I.U.) and Intermedin (133.3 Phoxinus units daily)	4N	3	1.86 ± 0.0213	Depressed
	4H	4	1.10 ± 0.0192	Atrophy
Intermedin (133.3 Phoxinus units daily)	4N	4	1.89 ± 0.0245	Depressed
	4H	3	1.16 ± 0.0156	Atrophy
Adrenal Cortical Extract (=2.5 g. beef cortical tissue daily)	4N	3	1.79 ± 0.0211	Depressed
	4H	3	1.12 ± 0.0159	Atrophy

[1]N: Normal goldfish; H: Hypophysectomized goldfish.

[2]Based upon data presented by Chavin (1956).

[3]For definition of terminology see text.

[4]The first statistically significant depression in epithelial height occurs two days after saline immersion to the figure indicated. The mean height gradually increases over a period of seven days to the normal range on the ninth day.

are also due Dr. Irby Bunding of the Armour Laboratories, Chicago, for supplying ACTH Lot 146-RS3 and ACTH-TSH Lot 84-5U2S2; Dr. Henri Choay of the Laboratoire Choay, Paris, for the intermedin; and Dr. David Klein of the Wilson Company, Chicago, for the adrenal cortical extract.

Results

In 85% of the 153 goldfish examined, irregularly round to oval follicles filled with colloid were found in the lymphoid tissue of the head kidney. The number of follicles varied from two to several hundred per animal, and was not correlated with experimental treatment, sex or level of gonadal maturity. The scattered and separate thyroid follicles of the goldfish were found in the throat, clustered for the most part about the origin of the first and second afferent branchial arteries (Chavin, 1956). In 77% of the control animals, follicles were present in the head kidney. Such follicles closely resembled those of the thyroid in histological structure, but were unencapsulated. The slightly basophilic cuboidal epithelial cells varied somewhat in size and shape, and their lateral cytoplasmic limits were indistinct (Plate I, Fig. 1). Nuclear shape was variable. The follicular colloid was homogeneous and stained with eosin and fast green.

Follicles in the head kidney occurred in 87% of the experimental fish. The number of animals containing such follicles in each group is indicated in Table 1. The epithelial heights of these follicles varied with the experimental procedure

to which the animals were subjected, Table 1. Immersion in hypertonic saline produced a temporary depression of epithelial cell height after two days. At this time, there was increased cytoplasmic basophilia with little or no vacuolation. The nuclei were oval and deeply basophilic (Plate I, Fig. 2). Epithelial height and cell morphology returned to the normal state by the ninth day of treatment. Hypophysectomy produced atrophy of the follicular epithelium. The basophilic nuclei appeared as flattened bulges in the attenuate cells, (Plate I, Fig. 3). The epithelium of hypophysectomized fish was not altered by the administration of isotonic saline, ACTH, optic lobe of the brain, head kidney or opisthonephros or by saline immersion. These treatments, however, depressed the follicular epithelial height in normal fish. In both the hypophysectomized and normal animals, implantation of pituitary tissue or injection of purified ACTH with TSH elicited epithelial hypertrophy. The now deeply basophilic and columnar epithelial cells contained many coarse granules and several large chromophobic vacuoles (Plate I, Fig. 4). The large, vesicular nuclei each contained a prominent nucleolus. The follicular colloid was usually vacuolated.

Discussion

The histologic findings indicate that the follicles in the head kidney of the goldfish are structurally similar to the thyroid tissue in the throat. In addition, the responses of the head kidney follicles to various physiologic alterations are identical with those of the throat thyroid. It is reasonable, therefore, to conclude that the above-described structures in the head kidney are functional thyroid follicles.

The origin of the thyroid tissue in the head kidney is an interesting problem. The presence of follicles in a lymphoid organ a distance from the normally located gland suggests their metastatic origin. As the first lymphoid organ in the venous drainage of the head, the head kidney is a filter in which the blood-borne cells may lodge. In addition, the absence of the connective tissue membrane (normally found around each thyroid follicle) about the head kidney follicles may be an indication of their migratory origin. On the other hand, the ontogeny of the thyroid suggests an alternate means by which the follicles may appear in the head kidney. The teleost thyroid originates as a median ventral outgrowth from the pharygeal floor in the region of the second pair of gill pouches. This diverticulum grows posteriorly and eventually comes to lie anterior to the heart, but with further development it spreads anteriorly until the adult condition is attained (Maurer, 1886; Gudernatsch, 1911). If some of the cellular precursors of the thyroid deviate slightly to proliferate in a dorsal direction, the eventual appearance of thyroid follicles in the head kidney may result. In the discussion following the paper of Gorbman (1955), it was suggested that the atypically-located thyroid tissue in the platyfish is neither of teratological origin nor derived from mesonephric blastema. This suggestion is supported by the results of the present study, for the majority of goldfish, normal in all observed histological aspects, contained thyroid tissue a considerable distance from the normal locus in material derived from the pronephros. Thus the concept of metastatic or ontogenetic origin of the aberrantly located thyroid follicles, presented by Baker *et al* (1955) and Gorbman (1955), is favored at this time, although no direct evidence is on hand to support or deny any suggestion as to the origin of the described follicles.

Cellular proliferation may not always remain under the control of the mechanisms normally directing growth in fishes or other organisms. Such independent or random multiplication of cells which is later controlled, not at the cellular but at the tissue level, has been suggested to be part of a normal development pattern (Breder, 1952). The subsequent integration of the results of such atypical growth processes into the economy of the goldfish is indicated by the follicular organization of thyroid cells in the head kidney and the similar reaction of these and normally located cells to various stimuli. Because of this developmental pattern, the goldfish thyroid may prove to be an invaluable tool in the morphological, physiological and biochemical study of normal and atypical growth processes.

Summary

Thyroid follicles have been found in the lymphoidal pronephric remnants, the head kidneys, in 85% of 153 goldfish studied microscopically. These follicles are similar to those of the normally-located thyroid in structure and in reaction to various stimuli (hypophysectomy; saline immersion; implantation of pituitary, brain, head kidney or opisthonephros; injection of isotonic saline, ACTH, ACTH and TSH, ACTH and intermedin, intermedin, or adrenal cortical extract).

The origin of the aberrantly-located thyroid tissue is suggested to be a metastatic or ontogenetic phenomenon.

The utility of the goldfish thyroid in the study of normal and atypical growth processes is indicated.

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EXPLANATION OF THE PLATE

Plate I

Thyroid follicles in the head kidney of the goldfish, *Carassius auratus* (L.) Hematoxylin-eosin. 980×.

Fig. 1. Control goldfish.

Fig. 2. Depressed epithelial height in a normal goldfish immersed in 0.7% saline for two days.

Fig. 3. Atrophy of epithelium in a three-week postoperative, hypophysectomized goldfish.

Fig. 4. Epithelial hypertrophy in a hypophysectomized goldfish after administration of ACTH-TSH.